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Theme

Elaboration and characterization of thin film composite membranes

Presented by

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« I am among those who think that science has great beauty. A scientist in his laboratory is not only a technician: he is also a child placed before natural phenomena which impress him like a fairy tale. We should not allow it to be believed that all scientific progress can be reduced to mechanisms, machines, gearings, even though such machinery also has its beauty.

Neither do I believe that the spirit of adventure runs any risk of disappearing in our world. If I see anything vital around me, it is precisely that spirit of adventure, which seems indestructible and is akin to curiosity. »

Marie Curie

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Emy

Abbreviation list

AETMAC: 2-[(acryloyloxy)ethyl] trimethylammonium chloride

AI : Artificiel Intelligence

CA : Cellulose Acetate

CA : carbonic anlydrase

CAN : Ceric Ammonium Nitrate

CLEAs : cross linked enzyme aggregation

CNTs : carbon nanotubes

DMDMOS: dimethyldimethoxysilane

DRX : X-ray deffraction

GO : graphen oxide

HRP: horse radish peroxidase

IR : Infra-red

pH : Hydrogen Potencial

LGC : lipase extracted from watercress seeds

LGS : lipase extracted from sesame seeds

MA : Maleic Acid

MOFs : metal organic frame work

PES: Polyethersulfone

PSF : Polysulfone

PVDF : Poly Vinylidene Fluoride

PVA : Poly Vinyl Alcohol

PSSA : Polystyrene Sulfonic Acid

PVAc : poly(vinyl acetate)

PTFE: polytetrafuoroethylene

PMMA: poly(methyl methacrylate)

PLE: pig liver esterase

PM : particulete matter

TMOS: tetramethxysilane

UV : ultaraviolet radiation

Abstract

This work aims at the elaboration of synthesis of the membranes based on polymers and lipases of a vegetable nature (crude extracts of oil seeds: watercress and sesame seeds) to be used as biocatalysts with deferent fractions in order to optimize it. This thin film composite membrane was carried out with PVA (Polyvinyl Alcohol) and PSS (polystyrene sulfonate) in the presence of vegetable lipases. In order to study the characterization of the resulted thin films several technics are used like Ultraviolet-visible spectroscopy, X-ray diffraction, Fourier Transform Infrared Spectroscopy in FTIR transmission and optical microscopy. The objective of this combination of biocatalytic functionalities with PVA membranes has led to the development of biocatalytic PVA membranes, which synergistically harness the desirable properties of PVA and the selective catalytic activity of embedded biocatalysts.

Key words: Biocatalysis, polymer membrane, biocatalytic membranes, PVA (Polyvinyl Alcohol), PVA biocatalytic membranes, immobilization.

Résumé

Ce travail vise l'élaboration de la synthèse des membranes à base de polymères et de lipases d'origine végétale (extraits bruts de graines oléagineuses : cresson et graines de sésame) à utiliser comme biocatalyseurs avec différentes fractions afin de les optimiser. Cette membrane composite en film mince a été réalisée avec du PVA (l'alcool polyvinylique) et du PSS (polystyrène sulfonate) en présence de lipases végétales. Afin d'étudier la caractérisation des films minces obtenus, plusieurs techniques sont utilisées, telles que la spectroscopie ultraviolet-visible, la diffraction des rayons X, la spectroscopie infrarouge à transformée de Fourier en transmission FTIR et la microscopie optique. L'objectif de cette combinaison de fonctionnalités biocatalytiques avec des membranes de PVA a conduit au développement de membranes de PVA biocatalytiques, qui exploitent de manière synergique les propriétés souhaitables du PVA et l'activité catalytique sélective des biocatalyseurs intégrés.

Mots-clés : Biocatalyse, membrane polymère, membranes biocatalytiques, PVA (l'alcool polyvinylique), membranes biocatalytiques en PVA, immobilisation.

ملخص

يهدف هذا العمل إلى إعداد أغشية قائمة على البوليمرات والليبازات ذات الطبيعة النباتية (المستخلصات الخام لبذور الزيت: الجرجير وبذور السمسم) لاستخدامها كعوامل حيوية مع أجزاء مختلفة من أجل تحسينها. تم إعداد هذا الغشاء المركب الرقيق باستخدام "بولي فينيل الكحول" و "بوليمار ستيرين سلفونات" بوجود الليبازات النباتية. لدراسة خصائص الأفلام الرقيقة الناتجة، تم استخدام تقنيات عدة مثل الطيف الضوئي المرئي فوق البنفسجي، حيود الأشعة السينية، الطيف الكهرومغناطيسي بالأشعة تحت الحمراء، والميكروسكوبية الضوئية. الهدف من هذا الجمع بين الوظائف الحيوية التحفيزية وأغشية "بولي فينيل الكحول " أدى إلى تطوير أغشية "بولي فينيل الكحول" الحيوية التحفيزية، التي تستفيد بشكل متكامل من الخصائص المرغوبة لـ "بولي فينيل الكحول" والنشاط التحفيزي الانتقائي للعوامل الحيوية المدمجة .

الكلمات المفتاحية: التحفيز الحيوي، غشاء البوليمر، الأغشية التحفيزية الحيوية، بولي فينيل الكحول، الأغشية التحفيزية الحيوية لبولي فينيل الكحول، التثبيت .

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Summary

General Introduction

Over the past two decades, biocatalysis has advanced into a mature and extensively utilized technology across various sectors [1]. In the early 2000s, with few notable exceptions, its application remained largely confined to niche areas, primarily focusing on the synthesis and resolution of optically active intermediates. Since then, the field has progressively transformed into a broadly applicable platform for chemical synthesis and industrial manufacturing, as extensively documented in the literature. This evolution has been largely driven by the accelerated discovery of novel enzyme variants, facilitated by advancements in bioinformatics and enzyme engineering supported by computational modeling [2]. Among the innovative techniques currently incorporating biocatalysis, membrane preparation has gained significant attention for its potential in advanced material development.

In many industrial and environmental application, the use of membranes has become essential for efficient separation process. This technique plays a crucial role in filtering, purifying, and concentrating specific components in gases and liquids. Traditional membrane technology has provided considerable insight into separation and reaction. However, many researchers now agree that new advances in membrane technology will require synergy between the strength and processing properties of traditional polymeric membranes and the selectivity. From this dimension appears new generation of membranes, so called, the biocatalytic membranes. Biocatalytic membranes are entities in which an enzymatic catalysis integrates with membrane separation, offering enhanced efficiency in environmental remediation, carbon capture, and industrial processes [3]. Among the various materials used for membrane fabrication, polymer membranes are especially valued for their tunable physical and chemical properties, mechanical stability, and ease of functionalization. Among all potential candidates, one that meets the requirements is poly (vinyl alcohol) (PVA), a polyhydroxy polymer that is widely used for packaging and producing polarizer films. PVA membranes have a good film-forming capacity, relatively low production cost, wide-ranging crystallinity, complete biodegradability, crystal modulus and a considerable amount of hydroxyl groups that serve as a proton transfer bridge [4].

PVA-based membranes are becoming more competitive due to two essential characteristics, namely, proton conductivity and methanol crossover. This polymer can be modified through 3 methods: Copolymerization, Crosslinking and Blending. Integrating biocatalysis within PVA membranes, due to their biological nature, are susceptible to being inactivated under aggressive process conditions. This represents a challenge for its industrial application that can be overcome using immobilization techniques. Different immobilization methodologies can be classified into physical,

physical/chemical, and chemical methods. Among the physical techniques is adsorption, which consists of binding the enzyme to the support utilizing weak bonds such as those of van der Waals and hydrophobic support/enzyme interactions. Unlike adsorption methods, chemical methods, help prevent the enzyme from leaching off the support material, thereby enhancing the biocatalyst's stability. These methods include covalent bonding between the enzyme and the support as well as cross-linked enzyme aggregates, where enzyme molecules are covalently bonded to each other using a chemical cross-linker. This technique does not require a solid support; however, CLEAs are often immobilized on solid carriers, in order to enhance their mechanical strength and make them suitable for use in enzymatic bioreactors.

Therefore, this work aims to extract lipases from seeds in order to use them as biocatalysts in thin film composite membrane elaboration. After the **general introduction**, a bibliographical overview of biocatalysts and polymer membrane, focusing on PVA membranes will be the subject of the **first chapter**. In the **second chapter**, our discuss is about the **experimental part** in which the operating protocols and the equipment used are presented. The **third chapter** of this dissertation devoted to the results and discussion relating the characterization methods of the thin film composite membrane prepared in the presence of the prepared lipases of plant origin (from seeds). Finally, we conclude this manuscript with a **general conclusion** to highlight the results of this work, the objectives achieved and perspectives.

Chapter 01 : Biocatalysts and Polymer Membranes

1. Introduction

Biocatalysis, using enzymes as natural catalysts, is a growing field bridging biotechnology and sustainable chemistry. Its high specificity, efficiency, and eco-friendliness make it a preferred alternative to traditional chemical catalysis, especially for greener, resource-efficient industrial processes. Enzymes function under mild conditions, offer high selectivity, and can be engineered or immobilized to improve stability and reuse, benefiting applications from pharmaceuticals to biofuels.

Advances in enzyme discovery, engineering, and immobilization have expanded biocatalysis into industrial and biorefinery applications. However, maintaining enzyme activity and enabling reuse remain challenges, where polymeric membranes—particularly polyvinyl alcohol (PVA) membranes—play a vital role. These membranes act as supports and functional interfaces, enhancing enzyme stability and process efficiency. Techniques like aqueous phase separation allow enzyme integration without harmful solvents, making biocatalytic membranes promising for water treatment, chemical synthesis, and more.

2. Biocatalysis: Fundamentals and Applications

2.1. working principle of biocatalysis:

Biocatalysis stands as a fundamental concept within the realm of biotechnology, drawing from a wide array of scientific disciplines [5] to enable environmentally friendly and efficient chemical transformations. At its core, biocatalysis involves the use of biological catalysts typically enzymes or microorganisms to perform chemical reactions [6]. These catalysts are prized for their high specificity and efficiency [6], [7], often operating under mild conditions that reduce energy consumption and minimize the generation of hazardous waste [6], [8]. Thus, biocatalysis not only exemplifies the convergence of biotechnology and green chemistry but also reinforces the shift toward more sustainable chemical manufacturing.

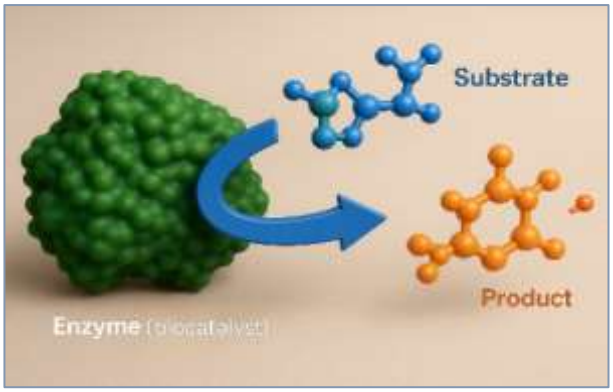


Figure I. 1: *working principle of biocatalyst (Generated by AI)*

Biocatalysts include enzymes, microorganisms, and even inorganic colloids, each with specific functions [5], [7], [9]. Enzymes, derived from natural resources or produced recombinantly, catalyze various reactions with high selectivity [7],[10]. Microorganisms, such as ciliates, can be used for biotransformation of sterol metabolites [5]. Inorganic nano-sized colloids participate in electron transfer during biological redox reactions [9]

2.2. Advantages of biocatalysis over traditional chemical catalysis

Traditional chemical catalysis relies on synthetic or inorganic catalysts that often require harsh conditions like high temperature, pressure and organic solvents, which can lead to lower selectivity and more side reaction. In contrast, the sustainable nature of biocatalysis is rooted in its ability to reduce environmental impact. It achieves this through the use of renewable raw materials, the minimization of toxic byproducts, and reaction conditions that are generally safer and less energy-intensive than traditional chemical processes and more details breakdown of the advantages are listed in the table below. Furthermore, advancements in molecular biology and biotechnology, particularly in the field of protein engineering, have significantly expanded the capabilities of biocatalysis. These developments have enabled scientists to not only optimize natural enzymes but also design novel catalysts tailored for specific reactions [7], [11]. Furthermore, advancements in molecular biology and biotechnology, particularly in the field of protein engineering, have significantly expanded the capabilities of biocatalysis. These developments have enabled scientists to not only optimize natural enzymes but also design novel catalysts tailored for specific reactions [7], [11].

Table I. 1: Advantages of biocatalysis over chemical catalysis

Criteria	Biocatalysis	Traditional chemical catalysis	Ref
Efficiency	Highly efficient, operates under mild conditions, reducing steps and by products	Less efficient, often requires harsh conditions and multiple steps	[12]
Reaction specificity	Highly specific, ensuring precise reactions with minimal waste.	Less specific, leading to more by products and waste.	[13]
Environmental impact and sustainability	Eco-friendly, uses renewable sources, reduces energy consumption, and toxic waste.	High environmental impact due to harsh chemicals and energy consumption.	[14]
Cost effectiveness	Economically advantageous due to reduced energy and waste disposal costs.	Higher operational costs due to energy consumption and waste management.	[15]
Operational conditions	Operates under mild conditions (temperature, pH)	Requires extreme temperatures and pressures.	[16]
Innovation and development	Continuous R&D leads to discovering and optimizing new enzymes for biocatalysis and processes, driving innovation.	Slow innovation due to the complexity and cost of developing new chemical catalysis and processes.	[17]

2.3. Application of biocatalysts:

Biocatalysts have widespread applications in diverse fields such as organic synthesis[18], environmental remediation [19], and diagnostic technologies [20]. Enzymes, in particular, offer distinct advantages over conventional inorganic, metal-based, or chemical catalysts. When immobilized onto porous supports, enzymes can facilitate the production of high-purity, high-value products. Additionally, their use significantly reduces the occurrence of side reactions and simplifies downstream processing by easing post-reaction separation problems [14]. In Table 2, the most specific industrial applications of enzyme are reported [21], [22] [23].

Table I. 2: biocatalysis application at the industry and their classes [22]

Industry	Enzyme class	Application
Pulp and paper	Lipase	Pitch control, contaminant
	Protease	Biofilm removal control
	Amylase	Starch-coating, de-inking, drainage
	Xylanase	Bleach boosting
	Cellulase	De-inking, drainage improvement, fiber modification
Detergent	Protease	Protein stain removal
	Amylase	Starch stain removal
	Lipase	Lipid stain removal
	Cellulase	Cleaning, color clarification, anti-redeposition (cotton)
	Mannanase	Mannanan stain removal
Fats and oils	Lipase	Transesterification
	Phospholipase	De-gumming, lyso-lecithin production
Organic synthesis	Lipase	Resolution of chiral alcohols and amides
	Acylase	Synthesis of semi-synthetic penicillin
	Nitrilase	Synthesis of enantiopure carboxylic acids
Food	Protease	Milk clotting, infant formulas (low allergenic), flavor
	Lipase	Cheese flavor
	Lactase	Lactose removal (milk)
	Pectin methyl esterase	Firming fruit-based products
	Pectinase	Fruit-based products
	Transglutaminase	Modify visco-elastic properties
Leather	Protease	Unhearing, bating
	Lipase	De-pickling
Baking	Amylase	Bread softness and volume, flour adjustment
	Xylanase	Dough conditioning
	Lipase emulsifier	Dough stability and conditioning
	Phospholipase	Dough stability and conditioning
	Glucose oxidase	Dough strengthening
	Lipoxygenase	Dough strengthening, bread whitening
	Protease	Biscuits, cookies
	Transglutaminase	Laminated dough strengths

2.4. Advantages and limitations of biocatalysts applications

As a green and sustainable technology, biocatalysis plays a crucial role in the production of pharmaceuticals and various commodity chemicals, demonstrating both ecological responsibility and industrial relevance [11].

Enzymes, the most common biocatalysts, offer several advantages. Their inherent selectivity (chemo-, regio-, and stereoselectivity) ensures that reactions yield specific products with high purity and minimal side products [6]. Such selectivity is especially valuable in the synthesis of complex pharmaceuticals, where precision and efficiency are paramount. The alignment of biocatalytic processes with the principles of green chemistry and sustainable development underscores their growing importance in both academic research and industrial applications [8], [11].

Despite its many advantages, biocatalysis is not without challenges. One of the main obstacles lies in the incompatibility of reaction conditions between chemical and enzymatic steps, which can hinder the efficiency of multistep synthesis [7]. However, these limitations are being actively addressed through strategies such as compartmentalized reaction systems and the use of flow chemistry, which help to maintain optimal conditions for each reaction step [7].

2.5. Emerging Trends and Innovations of Biocatalysis in Sustainable Chemistry

Biocatalysis represents a powerful and evolving tool for sustainable chemistry [11], [24]. By integrating diverse scientific disciplines and leveraging advances in biotechnology, it enables the efficient and environmentally responsible production of valuable chemicals and pharmaceuticals [5],[6]. Recent trends in the field highlight the dynamic and innovative nature of biocatalysis. Discoveries of novel enzymes, the development of enzyme cascade reactions, and the integration of intelligent manufacturing technologies have all contributed to the expansion of biocatalysis into new and more complex chemical territories [24]. The rise of continuous flow systems, coupled with process analytical technologies and digital tools, further underscores the modern direction of the field [7]. To enhance the performance and economic feasibility of biocatalytic processes, several strategies have been employed. Innovations in substrate selection, medium optimization, and reactor design contribute to improved reaction efficiency [11]. Enzyme immobilization, for instance, not only enhances enzyme stability but also facilitates their reuse, reducing operational costs and improving overall process sustainability [11]. Additionally, biocatalysis can be effectively combined with other catalytic methods, such as homogeneous and heterogeneous catalysis, to create hybrid systems that offer greater flexibility and functionality [25]. Continued innovation in enzyme engineering, process optimization, and system integration will undoubtedly enhance the scope and impact of biocatalysis, solidifying its role at the forefront of green chemistry and industrial biotechnology [11][7].

3. Membrane Polymers: Characteristics and Role in Biocatalysis

3.1. General properties of polymeric membranes:

Polymeric membranes are extensively utilized in separation technologies owing to their adaptable physicochemical properties, which greatly affect their performance in applications like water treatment, gas separation, and biomedical fields. A key property of polymeric membranes is actually their selective permeability, and that enables the differential transport of molecules in terms of size exclusion, charge interactions, or in their affinity, thereby easing effective separation processes [26]. The permeability and selectivity of these membranes are linked to their microstructure. This includes porosity and pore size distribution, which govern transport dynamics [27]. Another necessary attribute, being mechanical stability, is such that membranes must withstand operational pressures and mechanical stresses without any deformation or failure; this depends upon the polymer's molecular structure and the additional substrate and processing conditions [28]. Chemical resistance to solvents, acids, as well as bases ensures membrane durability in harsh environments, expanding their applicability across diverse industrial sectors [29]. Thermal stability defines, in addition, the upper limits for membrane operation, with temperature tolerance closely related with the polymer's glass transition temperature and crystallinity [30]. Additionally, surface properties such as hydrophilicity or hydrophobicity considerably affect fouling tendencies as well as interactions with permeating species, impacting long-term performance [31]. Thus, the interplay amongst these properties must be optimized to tailor polymeric membranes for certain specific applications, balancing permeability, selectivity, stability, and operational longevity.

3.2. Common polymer materials used in membrane fabrication:

Polymeric membranes constitute a critical component in various separation processes, with their performance largely determined by the intrinsic properties of the polymer materials employed. Common polymers used in membrane fabrication include polysulfone (PSf), polyethersulfone (PES), polyvinylidene fluoride (PVDF), polyvinyl alcohol (PVA), and cellulose acetate (CA), each chosen for its chemical resistance, mechanical strength, thermal stability, and hydrophilicity. PSf and PES are prominent because of the superb thermal and chemical stability these possess, making these suitable to ultrafiltration and microfiltration applications, as PVDF is valued because of its hydrophobicity and robustness within harsh chemical environments [32] [33]. PVA have a wide use as a result of their hydrophilic nature, and also high selectivity, specifically within nanofiltration with pervaporation, and crosslinking strategies get employed for enhancement of their chemical and mechanical durability

[34][35]. Cellulose acetate remains relevant, in reverse osmosis membranes especially, due to its superb film-forming, biodegradability properties. Its operational lifespan is limited by its susceptibility to hydrolysis, though [36]. More recent trends do also highlight further development in polymer blends as well as nanocomposites for improved membrane fouling resistance in addition to separation efficiency, thus showing a more continued evolution for polymer materials of membrane science [37].

3.3. Polymer based membranes in biocatalytic system:

Polymer membranes play an indispensable role in biocatalytic applications by enabling effective enzyme immobilization, enhanced stability, and improved substrate-product separation. Immobilizing enzymes on or inside the polymer membranes maintains catalytic activity, and facilitates the reuse and continuous operation of enzymes [38]. Polymer membranes possess selective permeability, which enables substrates to pass through and enzymes and larger biomolecules to be retained, thus enhancing the efficiency of the reaction and preventing enzyme loss [39].

More recently, membrane materials have also been further enhanced through the creation of functionalized polymer membranes with tailored surface chemistries to promote enzyme affinity while maintaining fouling resistance[40]. Membrane bioreactors combine catalysis and separation in one unite and leverage these membranes for continuous processing with high product purity and process intensification [41]. Furthermore, Polymer membranes with controlled pore size distribution also enable spatial organization of enzymes, promoting multi-enzyme cascade reactions by avoiding cross-inhibition and enhancing overall catalytic activity [42]. These developments underscore the vital role of polymer membranes in improving the efficiency, sustainability, and scalability of biocatalytic processes.

3.4. Role of membranes in enhancing biocatalytic efficiency (selectivity, stability, recyclability):

Membranes play a pivotal role in enhancing biocatalytic efficiency by providing structural frameworks that improve enzyme performance across multiple dimensions (selectivity, stability, and recyclability). When enzymes are immobilized within or on the surface of membranes, reactions can be more precisely controlled. This led to minimizing unwanted side reactions and allows for the efficient removal of products, which helps prevent enzyme inhibition and reduces byproduct formation [43] membrane can be tailored, for instance, polyelectrolyte-doped hollow nanofibers allow positional assembly of multiple enzymes for cascade reactions at oil-water interfaces, demonstrating up to 114.45-fold enhancement in reaction rates compared to free enzyme systems [44]. Which prove the enhancement of the interaction between the enzyme and its support. This enzyme-support interactions not only boost catalytic activity but also offer precise control over reaction rates and substrate access

[43], [45]. Additionally, these immobilization strategies enhance enzyme stability by protecting it against denaturation and leaching, resulting in longer-lasting activity and better stability over repeated use and continuous operation.

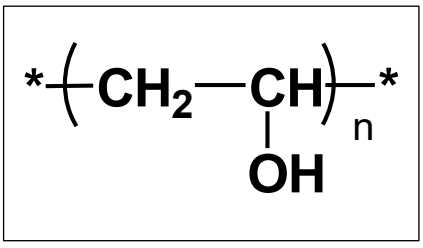
Membrane systems also substantially improve enzyme stability through diverse mechanisms: protection from harsh external conditions, prevention of enzyme aggregation, and creation of favorable local environments [46][44] . The immobilization of enzymes within or onto membrane structures enhances thermal, chemical, and operational stability compared to their free counterparts [47][46]. Notably, enzyme recyclability is dramatically improved through membrane-based immobilization, enabling multiple reaction cycles without significant activity loss [48]. Advanced membrane designs, such as hybrid nanocontainers with selective permeability [49], multi-shelled metal-organic frameworks, and supported liquid membranes based on ionic liquids [50], further enhance these advantages by providing tailored structures that control mass transport while maintaining catalytic activity.

These membrane systems represent a significant advancement in sustainable biocatalysis by addressing key industrial requirements for enzyme performance, including reduced biocatalyst costs through reusability and improved process economics through enhanced selectivity and stability [46][44]. Thus, the integration of membranes in biocatalytic systems offers a robust platform for advancing efficiency, selectivity, stability, and recyclability in both laboratory and industrial applications.

4. PVA Membranes: Structure, Properties and Applications

4.1. Chemical composition and structural characteristics of PVA membranes:

Among the polymers, Poly(vinyl alcohol) (PVA), a polyhydroxy polymer extensively utilized in packaging and the manufacture of polarizer films, has garnered significant interest from researchers due to its superior film-forming capabilities, high hydrophilicity, commendable physical and chemical stability, affordability, and commercial accessibility [4].



Schema I. 1: Structure of PVA

PVA as shown in Figure 1 is an excellent biodegradable and water-soluble polymer. In contrast to the majority of vinyl polymers, PVA is produced by a variety of indirect techniques that include the alcoholysis (also known as saponification or hydrolysis) of polyvinyl acetate rather than directly polymerizing the equivalent monomer. Strong acids or bases can catalyze the conversion process [4].

Nevertheless, the PVA membrane has inadequate stability in aqueous solutions due to its strong affinity for water, which induces swelling of the membrane and adversely impacts its selectivity. For this

reason, the PVA membranes should be modified for forming more durable membranes [51]. There are several methods to crosslink the PVA such as heat treatment, irradiation, and reaction with multifunctional compounds. Among them, the crosslinking by chemical reagents is a preferential adoption since a capability to tailor the morphology and the physicochemical properties of the PVA matrix can impact the separation performance of the membrane. The mechanical, swelling properties, morphology, and pervaporation performance of the PVA membrane are dependent on types of crosslinkers, crosslinking degree, PVA concentration and crosslinked conditions [52].

4.2. Hydrophilicity, biocompatibility, and mechanical properties:

PVA is a water-soluble polymer that has unique properties such as good cohesion, biocompatibility, biodegradability and hydrophilicity [53]. Hydrophilic polymeric membranes are suitable for selective removal of water from organic solvent (i.e., pervaporation dehydration of solvent). The hydrophilicity favors preferential sorption towards water over organic solvent and the diffusivity of water is faster than organic solvent with larger molecular size. One of the first commercialized pervaporation membrane materials is PVA, which remains the benchmark polymer of hydrophilic membranes for solvent dehydration [54]. The hydrophilicity of PVA comes from the pendant hydroxyl groups while semicrystalline structure is resulted from the two carbon atoms in the backbone of PVA [55].

The oleophobic nature of PVA minimizes the rate of fouling and because of this property, PVA can be used for the surface modification of the membrane. However, due to the hydrophilic nature, the excessive immersion of PVA in a solution can result in swelling of supporting membrane and deteriorate the filtration performance [56]. To balance the hydrophilic and hydrophobic properties of the membrane and lessen swelling, the PVA can be crosslinked using multifunctional substances like glutaraldehyde (GA) [57]. Also, combining PVA with nanoparticles like titanium dioxide (TiO2) may enhance structural and antifouling as well as structural properties of a membrane [57]

Blending PVA with other hydrophilic polymers with less compact structure (e.g., chitosan [58], CS[59]; sodium alginate, SA [60], [61]) was demonstrated to reduce the crystallinity of PVA and thus improve the permeation flux of PVA membrane.

Polyvinyl alcohol (PVA) membranes are widely recognized for their excellent biocompatibility, which makes them highly suitable for various biomedical and biocatalytic applications. PVA's hydrophilic nature, non-toxicity, and biodegradability contribute to its favorable interaction with biological tissues and cells, minimizing inflammatory responses and cytotoxicity [62]

Moreover, PVA hydrogels show excellent hemocompatibility, making them promising for vascular grafts and hemodialysis membranes due to their ability to reduce blood coagulation and

hemolysis [63]. The polymer's abundant hydroxyl groups allow chemical modifications and crosslinking, enabling the tailoring of mechanical properties and biofunctionality while maintaining cytocompatibility [64]. These characteristics have led to PVA's extensive use in wound dressings, artificial tissues, and enzyme-immobilizing membranes, where biocompatibility is critical for long-term performance and integration with living systems [65]. Overall, PVA membranes combine mechanical robustness with excellent biocompatibility, supporting their role as versatile biomaterials in advanced biomedical and biocatalytic membrane technologies.

4.3. Chemical modifications to enhance PVA membrane performance:

Unmodified PVA membranes frequently encounter issues such as inadequate mechanical strength, restricted water resistance, and elevated crystallinity [66]. To overcome these limitations, various chemical modifications have been developed to enhance PVA membrane performance. The main PVA modifications highlighted [67]are as follows:

1. **Copolymerization**: Coupling can be achieved by hydrophilic and hydrophobic copolymerization or through a chemical grafting-induced method by irradiation or chemical activation.
2. **Crosslinking**: Crosslinking of PVA can be achieved in many ways, such as freezing, heat treatment, irradiation and chemical treatment.
3. **Blending**: Blending of PVA can be realized with various sulfonated polymers (i.e. SPEEK, NafionTM, poly(styrene sulfonic acid) (PSSA) and PSSA-co-maleic acid (PSSA-MA)).

Graft copolymerization is an efficient way to combine the properties of two or more polymers into one unit. Natural polymers in their native form are generally unsuitable due to their high swelling and poor stability in biological environments [68]. A research platform for altering such traits and creating new polymeric materials with hybrid features is offered by graft polymerization. Thus, several studies have examined PVA graft copolymerization with various hydrophilic and hydrophobic monomers to improve physical, mechanical or biological properties [4]. According to Chen et al.,[69] ceric ion-induced redox initiated grafting is preferred in mild conditions with high graft yields and minimum side reactions.

Ceric ammonium nitrate (CAN) was used as a redox initiator to graft 2-[(acryloyloxy)ethyl] trimethylammonium chloride (AETMAC) onto polyvinyl alcohol (PVA). The study found that increasing AETMAC grafting from 10% to 40% significantly raised water absorption (from ~290% to ~588%) due to AETMAC's low crystallinity and hydrophilic nature, which enhanced the membrane's hydrophilicity. Additionally, Koohmareh et al. [70] developed a graft copolymer of styrene on PVA using reversible addition-fragmentation chain transfer (RAFT) polymerization, a controlled method that allows precise polymer synthesis with defined molar mass and low dispersity. Furthermore, Aoshima et

al. [71] introduced a coupling reagent based on partially hydrolyzed poly(vinyl acetate) (PVAc-OH) for synthesizing PVA graft copolymers via live cationic polymerization, resulting in grafted PVA with improved solubility in water, DMSO, and organic solvents like toluene and chloroform.

Polymer crosslinking alters key properties of polymers like PVA, including water uptake, swelling, thermal and mechanical stability, and acid retention [72]. Crosslinking generally reduces solubility by restricting polymer chain mobility, but excessive crosslinking can cause brittleness. Various crosslinking methods for PVA include heat treatment [73], irradiation [74], freeze-thaw cycles [75], and chemical treatment. Heat treatment increases crystallinity and decreases permeability and swelling with longer heating times. Microwave irradiation similarly affects PVA properties through vulcanization-like effects. Freeze-thaw cycling produces hydrogels with tunable tensile strength and swelling; increased cycles lead to denser polymer matrices due to higher crystallite formation. Chemical treatments using agents such as sulfosuccinic acid, glutaraldehyde, and others modify membrane chemistry. Ionic modification by adding ferric ions reduces crystallinity from 41.6% to 7.7% and increases tensile strength by 240% [66] . Acid treatments with orthophosphoric or sulfuric acid followed by epichlorohydrin crosslinking significantly affect swelling, mechanical stability, and ionic conductivity [76]. Blending PVA with polymers or nanomaterials, like coating polysulfone-nano ZnO membranes, enhances water treatment performance by improving rejection of TDS, COD, and ammonia [77].

Another study demonstrated improved anti-microbial properties and permeability in PVDF-PVA/GO composite membranes through silver (Ag) ion imprinting, which effectively inactivated 100% of Pseudomonas aeruginosa cells after 4 hours and reduce surface bacterial adhesion by approximately 94%, presenting promising applications in water purification processes [78].

In fuel cell applications, PVA modifications are crucial for enhancing ionic conductivity and methanol barrier properties. The incorporation of 2D nanoplatelets such as graphene oxide (GO) and its derivatives (porous GO and PEG-modified GO) into PVA matrices can significantly improve CO2 separation performance, with porous GO showing CO_2 permeance up to 607 GPU with a CO_2/N_2 separation factor of 36 at very low filler loadings (0.2 wt%) [79]. For high-temperature PEMFCs, acid-doped PVA-based membranes have been developed to operate at temperatures up to 200°C, offering advantages like high CO tolerance and simplified water management [80].

Beyond these approaches, PVA membranes can be further enhanced through crosslinking strategies and surface modifications to create hydrogels with tunable properties [81], [82]. The addition of plasticizers can improve flexibility and processability, while incorporating nanocellulose [83] or zinc oxide nanoparticles [84] can impart additional functionality such as antimicrobial properties.

4.4. Applications in biotechnology (Industrial and environmental), water purification, and biomedical engineering:

As a polymeric material, PVA possesses exceptional properties, such as excellent film-forming capacity, structural flexibility, hydrophilicity, and biocompatibility. Thus, PVA is widely used in various industrial applications [4], such as recovery of acids and valuable metals from waste solutions, membrane fuel cell applications, biological and biomedical applications, catalysis in organic and inorganic syntheses, and pervaporation separation of liquid mixtures.

Hülya Aykaç Özen et al.[85] explores the development of biodegradable hemp hurds/polyvinyl alcohol (PVA) composite membranes for particulate matter (PM) filtration, emphasizing sustainability and waste valorization. The study focuses on converting agricultural hemp waste into functional nanofiber membranes via electrospinning, addressing both environmental pollution and PM filtration challenges. The membranes combine PVA with hemp hurds (1–20 wt%) to create eco-friendly materials with enhanced filtration efficiency. These membranes are promising for: air purification system because of the effective particulate matter filtration in urban or industrial settings and in biodegradable packaging because of the dual-function materials for UV shielding and moisture control.

Furthermore, Qingchen Lu's [86]presents a straightforward, effective method to improve PVA membrane toughness by blending different molecular weights, yielding homogeneous membranes with superior mechanical and separation properties. This approach avoids complex chemical modifications while enhancing applicability in membrane technologies. The main application focus of the homogeneous toughened PVA blended membranes is in membrane separation technologies such as pervaporation, filtration, acid recovery, and potentially gas separation or fuel cells, where their improved mechanical properties and dimensional stability translate into enhanced performance and durability.

Moreover, PVA membranes, especially in the form of electrospun nanofibrous structures, show significant potential in water treatment applications. The PVA/GO@MOF membrane developed by Bin Xiang et al. [87] is a durable, high-performance membrane combining mechanical strength, underwater superoleophobicity, and fast photothermal self-cleaning. It achieves outstanding oily wastewater purification efficiency and organic pollutant degradation, with excellent reusability and resistance to harsh chemical and physical conditions, positioning it as a promising material for advanced wastewater treatment technologies.

In addition to the main application for solvent dehydration, hydrophilic pervaporation membrane can be coupled with reaction involving water as byproduct (e.g., esterification) where the *in-situ* removal of water could enhance the conversion rate [43]. Recently, catalyst was introduced into the PVA casting solution to prepare a catalytic membrane to further promote the reaction efficiency [88] . The reaction occurs with the aid of catalyst embedded in the membrane and meanwhile the byproduct water was

removed by the PVA-based membrane via pervaporation process. The potential in catalytic membrane would extend the application spectrum of PVA membranes from separation process into process intensification. [55].

5. Biocatalytic Membrane Polymers: Integration and Applications

5.1. Integration of biocatalysts into polymeric membranes:

The integration of biocatalysis and membrane technology has developed from simple sequential systems to sophisticated biocatalytic membranes capable of simultaneous reaction and separation, with ongoing research focused on improving performance and scalability for industrial biomanufacturing.
For polymer membranes, biocatalytic membranes combine both of enzyme functionality and separation properties [43]. Biocatalytic membranes offer numerus advantages to membrane technology owing to the diverse functionalities that biomacromolecules can impart to membranes [89]. For example, biocatalytic membranes employing hydrolase and oxidoreductase enzymes have been proposed for the elimination of micropollutants and the mitigation of fouling [89].

The idea of incorporating enzymes directly into polymer membranes to create biocatalytic membranes evolved later as membrane fabrication and enzyme immobilization methods matured. For example, recent advances like aqueous phase separation (APS) for membrane production have enabled easier incorporation of enzymes into polymer membranes, enhancing biocatalytic membrane functionality[90]

Currently, after the membrane fabrication, enzymes are typically immobilized on the membrane surface [43]. Several extra processing stages may be necessary for the immobilization of the enzyme. In addition, enzymes can lead to a decrease in membrane permeability and selectivity, enzyme immobilization or membrane production conditions can deactivate the enzyme, or enzymes can leach out of the membrane[90].

5. 2. Techniques for enzyme immobilization in polymer membranes:

Enzymes are commonly immobilized on solid carriers, membranes being one of the most versatile, to improve their stability, facilitate their reuse, and reduce overall processing and chemical conversion costs. Immobilization is achieved by fixing enzymes to or within solid supports, as a result of which heterogeneous immobilized enzyme systems are obtained [22].
Membranes, as enzyme carriers, can either be fabricated before (pre-existing) or during (formed in situ) the enzyme immobilization process [91]. On pre-existing (often commercially available) membrane

carriers, enzymes are immobilized through various mechanisms including adsorption, covalent bonding, and affinity binding [91].

Enzymes may be immobilized by a variety of methods as shown in Figure 3, which can be broadly classified as physical, where weak interactions between support and enzyme exist, and chemical, where covalent bonds are formed with the enzyme[92]. The table below mention the main chemical and physical methods of the enzyme immobilization.

Table I. 3: the difference between chemical and physical techniques of enzyme immobilization in polymer membranes.

Physical	Chemical
adsorption (physical, ionic) on a water-insoluble matrix, like mesoporous materials	covalent attachment to a water-insoluble matrix
inclusion (or gel entrapment)	cross-linking with use of a multifunctional
microencapsulation with a solid membrane	low molecular weight reagent and
micro-encapsulation with a liquid membrane	co-cross-linking with other neutral substances, e.g. proteins
formation of enzymatic Langmuir-Blodgett films	

The outcome is contingent upon the type of membrane support, including its hydrophilicity, hydrophobicity, reactive functional group density, porosity, and pore-size distribution. The dimensions of the (bio)catalyst(s), substrates(s), and products(s), as well as the chemical composition of the membrane and the solution, should be considered when selecting the matrix for enzymatic membrane reactors [93].

- **Entrapment:** The immobilization technique of entrapment relies on the positioning of an enzyme within the membrane. Asymmetric hollow fiber can provide an interesting support for enzyme immobilization [94]. To appropriately guide the creation of such systems, it is crucial to consider the quantity of biocatalyst loaded, its distribution and activity throughout the support, and its longevity. Immobilization of enzyme entrapment into membrane can be divided into 2 types, i.e. entrapment to membrane has been made and entrapment that coincide with membrane preparation. The membranes already used for this purpose are poly (vinyl alcohol) (PVA) – tetramethxysilane (TMOS), PVA – dimethyldimethoxysilane (DMDMOS), PVA-TMOS – DMDMOS, polystyrene, cellulose acetate (CA) – polytetrafuoroethylene (PTFE), PSf, PAN and poly(methyl methacrylate) (PMMA). Several enzymes, namely, lipase, b-glucosidase, glucose oxidase (GO), horse radish peroxidase (HRP), and pig liver esterase (PLE) have been immobilized using this technique [22].

In order to improve the stability and activity of lipase, Xu and Wang immobilized lipase into the gap formed between two kinds of membranes with different properties i.e. PVA-PTFE and CA-PTFE [95].

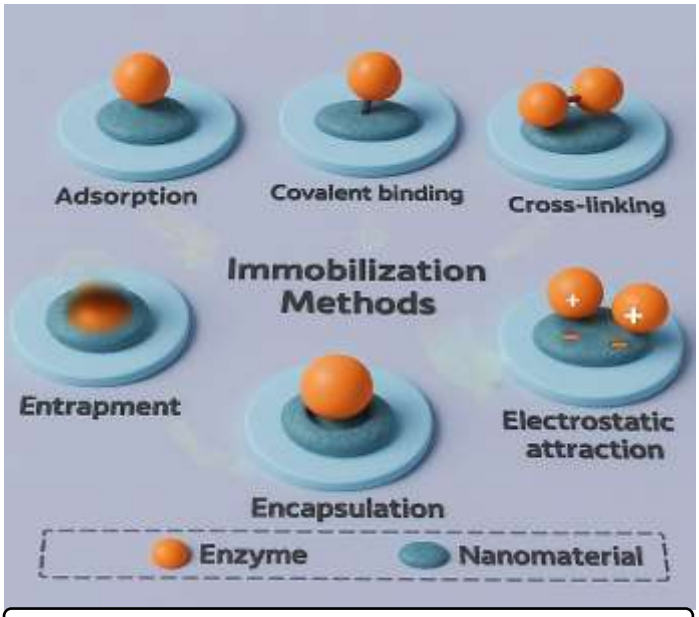


***Figure I. 2**: Immobilization methods in 3D (generated by AI)*

- **Covalent binding:** The foundation of the covalent binding technique is the covalent bonding between the membrane and the enzymes. Although they are not necessary for the catalytic activity, the functional groups that participate in this binding of enzymes to membranes can be amino, carboxyl, sulfhydryl, hydroxyl, imidazole, or phenolic groups [96].
- **Cross linking:** Enzyme immobilization can also be accomplished through intermolecular cross-linking of the protein to other protein molecules or functional groups on an insoluble support matrix. Cross-linking an enzyme with itself is both expensive and inadequate [96].
- **Affinity:** It is a method of immobilizing enzymes using a site-specific set of biomolecules. This approach allows you to manage the orientation of the biomolecule, avoiding enzyme deactivation and/or active site obstruction [22].
- **Adsorption:** Adsorption is the simplest way to immobilize the enzyme on the membrane. Adsorption is usually caused by variations in the membrane surface charge and the charge of the enzyme, which might result in a contact between the membrane and the enzyme. Adsorption often involves a weak interaction between the enzyme and the membrane [22].

5.3. Challenges of biocatalytic membrane systems:

Despite significant progress, there are many limitations to their production and widespread use and challenges remain, such as optimizing enzyme loading, maintaining long-term stability, and reducing preparation costs.

- **Trade-off between Enzyme Activity and Stability:** Noncovalent immobilization preserves activity but leads to leakage, while covalent bonding is stable but can reduce activity. Entrapment may cause fouling and affect stability. A denser sealing layer reduces leakage but hurts activity and stability due to mass transfer resistance [43].
- **Trade-off between Enzyme Loading and Membrane Permeability:** Increasing enzyme loading in the membrane pores decreases permeability, especially in reverse filtration where enzymes compact in pores. Higher pressure to increase flux can harm enzyme activity [43].
- **Issues with Multi-enzyme/Coenzyme Co-immobilization:** Immobilizing multiple enzymes/coenzymes on the membrane surface yields low loading and activity due to limited area. Entrapping them in pores by reverse filtration can lead to significant membrane permeability loss for liquid substrates[43].
- **Substrate Accessibility:** Large substrates struggle to reach enzymes immobilized within the membrane matrix, necessitating surface immobilization [43].
- **Difficulty Controlling Product Molecular Weight Distribution:** While "in-situ" removal is beneficial, obtaining a product with a narrow molecular weight distribution is hard due to uneven membrane pore size distribution, concentration polarization, and fouling [43].
- **Membrane Fouling:** Fouling by feed components, products, or intermediates impairs both separation and enzyme activity [43].
- **Challenges in Cleaning and Regeneration:** Standard chemical cleaning agents typically harm enzyme activity, and regeneration by reloading is required for reversible immobilization methods [43].

Due to the membrane's restricted specific surface area, low enzyme loading on the membrane results in a low catalytic efficiency, limiting its applicability in flow-through mode (short substrate retention period). In order to increase enzyme loading, some emerging nanomaterials, such as magnetic nanoparticles, carbon nanotubes (CNTs) [97], graphene oxides (GO) [98], and metal organic frameworks (MOFs)[99]. have been introduced into the membrane.

To address these issues and enable large scale industrial application, innovative strategies, including the use of biofunctionalized nanoparticles and advanced membrane materials are being explored.

5.4. Examples of biocatalytic polymer membranes in industry:

Protease, glucose oxidase, peroxidase, laccase, CA, formate dehydrogenase, formaldehyde dehydrogenase, alcohol dehydrogenase, β-galactosidase, dextranase, pectinase, and lipase are the most common enzymes for preparing biocatalytic membranes. The majority of these membranes have been applied to antifouling, water treatment, detection, and biomanufacturing. The desired configurations of biocatalytic membranes in diverse applications may differ due to the distinct needs for separation, catalysis, and mass transfer.

5.4.1. Biocatalytic membranes for water treatment:

Biocatalytic membranes based on crosslinked enzyme aggregates, as proven by Liu et al., provide a highly effective, stable, and reusable platform for removing micropollutants such as BPA and different dyes from water. Their high removal effectiveness, low salt rejection, and robust operating stability positions them as a leading technology in advanced water treatment applications [100], [101]. Biocatalytic membranes with crosslinked enzyme aggregates (CLEAs) are a sophisticated method for removing micropollutants from water that combines the selectivity and efficiency of enzymatic catalysis with membrane separation technology [102].

5.4.2. biocatalytic membrane and desalination:

Wenxiang Z. et al. presents an innovative approach for seawater treatment by integrating biocatalysts with biopolymer-based membranes. This research is done to enhance desalination and pollutant removal. The study emphasizes on the use of biopolymers such as chitosan, alginate, and cellulose derivatives as membrane matrices. The selection of these type of membranes is due to their biocompatibility, biodegradability, and functional groups that facilitate enzyme immobilization and stability in harsh marine environments. Electrospinning and phase inversion are the fabrication techniques highlighted for producing membranes with controlled porosity and surface properties, which are further improved by surface functionalization and incorporation of nanomaterials to boost mechanical strength and catalytic efficiency. Immobilized enzymes on these membranes effectively degrade organic contaminants and reduce biofouling, addressing key limitations in conventional membrane technologies [103].

5.4.3. biocatalytic membranes in biomedical engineering:

Applications of biocatalytic membranes are also suggested in biomedical engineering. Hybrid transplanted tissue survival containing polymer membranes is often hampered by the slow growth of blood vessels which may be remediated by incorporating proteins such as vascular endothelial growth factor in the membrane structure. In addition, biocatalytic membranes have also been suggested for use in dialysis[104].

5.4.4. Carbon Dioxide Capture and Conversion:

Biocatalytic polymer membranes are explored for CO_2 capture and conversion using enzymes like carbonic anhydrase (CA) or dehydrogenases. Janus biocatalytic membranes with differing hydrophobicities can be created, for instance, by coating a hydrophilic CNTs layer on a superhydrophobic polymeric MF membrane. Enzymes can also be coimmobilized within polymeric membranes for cascade reactions like the conversion of CO_2 to methanol [91].

6. PVA-Based Biocatalytic Membranes: Advances and Potential

6.1. Why PVA is an ideal matrix for biocatalytic membranes applications:

Polyvinyl alcohol (PVA) is a polymer frequently used as an ideal matrix for immobilizing various enzymes and cells for biocatalytic membrane applications due to its easy availability, low price, hydrophilic character and hydroxyl groups on the surface capable several key properties supported by academic research are listed bellow:

6.1.1. Excellent Enzyme Immobilization Capability: PVA membranes can effectively entrap enzymes such as glucoamylase, invertase, and cellulase while maintaining or even enhancing their activity and stability. For example, enzymes immobilized in UV-crosslinked PVA membranes showed improved thermal stability and favorable pH activity profiles compared to their free counterparts. The immobilized enzymes also exhibited good reusability and operational stability[105].

6.1.2. Hydrophilicity and Biocompatibility: PVA is highly hydrophilic, which facilitates substrate diffusion and interaction with immobilized enzymes, enhancing catalytic efficiency. Its biocompatibility ensures that enzymes retain their native conformation and activity when entrapped or immobilized within the polymer matrix [106].

6.1.3. Mechanical Strength and Tunable Properties through Crosslinking: Crosslinking PVA with agents such as glutaraldehyde allows tuning of membrane rigidity, porosity, and water uptake. Increased crosslink density enhances mechanical strength and stability, which is critical for long-term membrane operation in biocatalytic processes. However, it also affects proton conductivity and permeability, which can be optimized depending on the application [106].

6.1.4. Chemical and Thermal Stability: PVA membranes provide a stable environment that protects enzymes from denaturation caused by temperature or pH fluctuations. Studies have shown that enzymes immobilized on PVA membranes exhibit improved thermal and storage stability compared to free enzymes [106].

6.1.5. Ease of Fabrication and Versatility: PVA membranes can be fabricated using various methods, including UV irradiation and chemical crosslinking, allowing for easy incorporation of enzymes. They can also be combined with other materials like alginate and chitosan to further enhance immobilization and membrane performance [106].

6.2. Case studies and applications in wastewater treatment, biofuel production, and pharmaceutical industries:

Bhanupriya Das et al. presents a significant advancement in the development of cost-effective proton exchange membranes (PEMs) by using crosslinked polyvinyl alcohol (PVA) membranes instead of the typically expensive proton exchange membranes. The study tackles a common challenge in this field: finding materials that are both low-cost and effective. It demonstrates that these PVA membranes can conduct protons well, stay stable over time, and resist unwanted fuel crossover. What makes this research particularly interesting is its application in treating hemodialysis wastewater, transforming a challenging waste stream into a source of renewable energy. By exploring PVA as a simpler, cheaper alternative to conventional membranes. This research highlights the potential of PVA bio-catalytic membranes in bioelectrochemical fuel cells treating hemodialysis wastewater, emphasizing their viability as proton exchange membrane alternative. Overall, this research brings us closer to practical solutions that combine environmental care with energy innovation [107].

Another research from Vahideh Asadi and colleagues reports the development of biocatalytic composites where bovine carbonic anhydrase (BCA) is immobilized within zeolitic imidazolate framework-8 (ZIF-8) grown on cross-linked electrospun polyvinyl alcohol (CPVA) fibers. This design enhances enzyme stability against denaturants, heat, and acidic conditions compared to free or simply immobilized BCA. The composites demonstrated excellent reusability, maintaining significant catalytic activity over multiple cycles, and showed improved CO_2 sequestration by accelerating its conversion to calcium carbonate ($CaCO_3$). The fibrous structure allows easy recovery from reaction mixtures without complex separation methods, making these materials promising for industrial CO_2 capture applications under mild conditions. The study highlights that embedding BCA within ZIF-8 on CPVA fibers not only preserves enzyme activity but also improves operational stability and recyclability, with the BCA@ZIF-8/CPVA composite outperforming others in long-term activity retention and $CaCO_3$ precipitation efficiency [108].

6.3. Recent advances in PVA-based biocatalytic membranes:

Recent research has focused on enhancing the performance of PVA membranes by blending with other polymers, incorporating nanomaterials, and functionalizing the membrane surface to improve enzyme immobilization, substrate permeability, and antifouling characteristics. These modifications

have enabled PVA membranes to provide a favorable microenvironment for biocatalysts, improving enzyme stability and reusability in processes such as water treatment, CO_2 capture, and biosensing. For example, blending PVA with chitosan and starch has been shown to optimize hydrophilicity and selective permeability, which are crucial for efficient biocatalytic reactions [109]. Additionally, the integration of metal-organic frameworks (MOFs) and nanoparticles into PVA matrices has enhanced membrane selectivity and catalytic efficiency [108]. Moreover, in order to increase enzyme loading, some emerging nanomaterials, such as magnetic nanoparticles, carbon nanotubes (CNTs) [97], graphene oxides (GO) [98], and metal organic frameworks (MOFs)[99]. have been introduced into the membrane.

Despite these advances, challenges remain in scaling up production, improving long-term stability, and reducing fouling, which are active areas of ongoing research. Comprehensive reviews highlight the importance of tailoring polymer composition and membrane architecture to balance permeability, selectivity, and mechanical robustness for specific biocatalytic applications [105].

7. Conclusion:

Polymer membranes are ubiquitous in water purification and industrial separation techniques. In the face of global water shortages as well as developing biotechnology, the demand for more, better, and more versatile membranes continues to increase. In addition, there are investigations into making membrane production processes more sustainable. Biotechnology and membranes can be used for improving traditional production systems to maintain the sustainable growth of society. Typical examples include: new and improved foodstuffs, in which the desired nutrients are not lost during thermal treatment; novel pharmaceutical products with well-defined enantiomeric compositions; and the treatment of waste-water, wherein pollution by traditional processes is a problem. Polyvinyl alcohol (PVA)-based membranes have attracted significant attention in biocatalytic applications due to their excellent film-forming ability, hydrophilicity, chemical stability, and tunable mechanical properties and with the integration of biotechnology, all of the previous property will be enhanced. There are many enzymes which have been explored in membrane technology with the potential to provide interesting functionality. Despite all these advances challenges remains, the limited specific surface area of membranes leads to low enzyme loading and reduced catalytic efficiency, hindering their effectiveness in flow-through applications. To overcome this, nanomaterials like magnetic nanoparticles, carbon nanotubes, graphene oxides, and metal-organic frameworks have been incorporated into membranes. Additionally, innovative strategies such as biofunctionalized nanoparticles and advanced membrane materials are being developed to enhance performance and support large-scale industrial use.

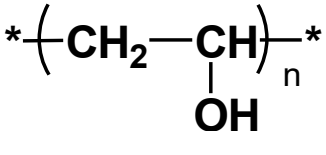
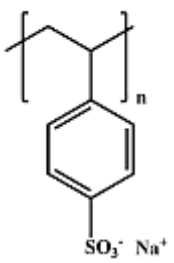
Chapter 02: Experimental part

In this chapter summarize the different materials, equipment, and protocols used for the various experiments and analyses performed. In particular, for the preparation of solution, polymer membranes, and their characterizations.

1. Chemicals and Biomass:

1.1. Chemicals :

- **Substrates:** polyvinyl alcohol (PVA), polystyrene sulfonate (PSS),
- **Solvents:** hydrochloric acid, acetone, water
- **Others:** glutaraldehyde**,**

	
PVA (polyvinyl alcohol)	PSS (Polystyrene Sulfonate)

***Scheme II. 1**: Chemical structure of PVA and PSS*

1.2. Biomass:

The two seeds used in this work (figure II.1), as possible source for the production of lipases which is the biocatalytic element used in films preparation, are available in the local market.

sesame seed 	Watercress seed 
Common name: sesame seeds **Scientific name**: *Sesamum indicum* **Family**: *Pedaliaceae*	Common name: passerage, cressonnette, nasitort, cardamine, cresson. Scientific name*: lepidium sativum linn* Family : *Brassicaceae*

***Figure II. 1**: seeds used for the extraction of lipases (biocatalyst)*

2. Material used:

In this work, several materials and devices were used to follow the different experiments carried out such as:

2.1. Precision balance:

All weighing were performed on a 0.0001g OAHUS precision balance (figure II.2).

***Figure II. 2**: OAHUS analytic balance*

2.2. Magnetic stirrer:

To carry out the mixtures of the membrane preparation, we used a plate with a magnetic stirrer from IKA RH basic 2. A magnetic stir bar was attached to the reaction system (figure II.3).

***Figure II. 3**: hot plate with a magnetic stirrer and a magnetic stir bar*

2.3. Lab Oven:

The glassware was dried using a MEMMERT universal oven (figureIII.4).

***Figure II. 4**: oven of the brand Universal MEMMERT*

2.4. Ultrasonic bath:

Ultrasonic water bath used for dispersion from SELECTA, ULTRASONS-HD marque. Ultrasonic cavitation generates high-speed liquid jets and mechanical stress that break apart particle agglomerates and disperse nanoparticles.

***Figure II. 5**: Ultrasonic bath from SELECTA, ULTRASONS-HD marque*

3. Methods:

3.1. Plant lipase extraction procedure:

Two different local oilseeds (watercress and sesame seeds) were used as a source of lipase. Obtaining the enzymatic extract (as shown in figure II.5) is generally done in 5 successive steps: soaking in water, germination, drying, grinding and then delipidation.

***Figure II. 6**: steps of lipases extraction of watercress seeds*

3.2. Membrane elaboration procedure:

Relaying on casting method, a series of nanocomposite films was prepared under the same operating conditions using different mass fractions of each type of nanofiller. Typically, PVA was solubilized in a volume of water while stirring at a temperature of 80°C. PSS was also dissolved in distilled water using magnetic stirring. At the same time, a desired amount of the enzymatic extraction (biocatalyst) was dispersed in the water. The three-solutions obtained were mixed while stirring at room temperature.

***Figure II. 7**: membrane elaboration procedure*

Nanocomposite films were also crosslinked using a glutaraldehyde (GLA) solution in the presence of HCL experimentally, 10 ml of GLA was poured over the already prepared dry films. The mixtures were then poured onto a silicon surface and spread to achieve a uniform thickness.

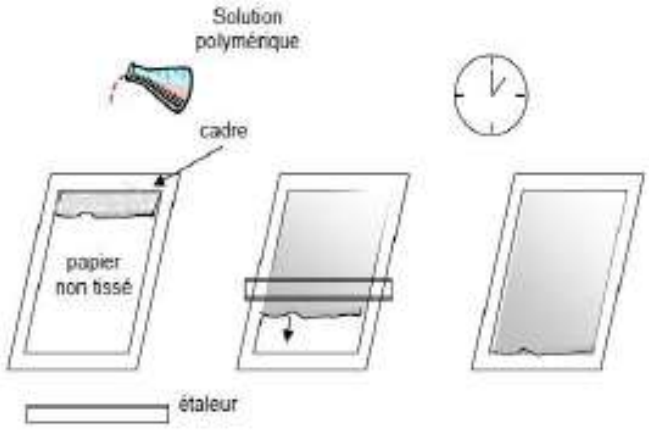


***Figure II. 8**: Way of pouring the mixture*

The films were then subjected to freezing and thawing cycles for a specific period; this step is key to removing excess solvent and giving the films flexibility. Finaly, drying at room temperature was performed to ensure complete evaporation of the solvent. The films thus obtained are characterized by FTIR, UV and by swelling.

***Figure II. 9**: pouring the mixture onto silicon surfac*

4. Characterization technics:

4.1. UV-visible spectroscopy:

UV-Visible spectrophotometry is a quantitative analytical method that measures the absorbance of a given chemical substance. In a molecule, UV-Visible electronic transitions involve the highest energies in chemistry (approximately 160 to 670 kJ mol-1). The order of magnitude of the energies involved is that of the binding energies of the molecules, and these rays can cause electronic transitions between the different energy levels of the molecules.

***Figure II. 10**: UVLine 9400 spectrometer*

- **Equipment :**

UV-Visible spectrophotometry analyses were performed at Setif University laboratory using an UviLine 9400 spectrometer.

4.2. Infrared analysis (FTIR):

Fourier transform infrared spectroscopy (FTIR) is based on the absorption of mid-infrared electromagnetic radiation by the sample to be analyzed. It allows, through the absorption of vibrations characteristic of chemical bonds, to determine the presence of certain chemical functions present in the material. The principle of this technique is based on the absorption of infrared radiation when the beam wavelength is equal to the vibrational energy of the molecule. The infrared range, between 4000 cm-1 and 400 cm-1, corresponds to the vibrational energy range of most molecules (mid-infrared). The result is a spectrum that provides a distinctive “chemical fingerprint” that can be used to visualize and identify organic and inorganic samples [110].

- **Equipment :**

Infrared spectrometry analyses were performed at Setif University laboratory using an Agilent Technologies Fourier transform infrared spectrometer. The spectra are collected and processed using Essential FTIR.

4.3. DRX (textural analysis):

X-ray diffractometry is an analysis technique based on X-ray diffraction by matter. The general method consists of bombarding the sample with X-ray, and analyzing the X-ray intensity which is diffused according to the orientation in space. The diffused X-rays interfere with each other, the intensity therefore presents maxims in certain directions, our subject is the phenomenon of “diffraction”.

***Figure II. 11**: Bruker D8 ADVANCCE ECO X-ray diffractor*

- **Equipment** :

The X-ray diffraction analyzes were performed at El-Tarf university laboratory on a *Bruker D8 ADVANCCE ECO*. The spectra are processed by P.D Analysis software.

4.4. Antibacterial Activity:

- **Equipment and Products for Antibacterial Effects:**

Table II. 1: Equipment and products used for antibacterial effects

Equipment	Products
Petri dishes, Metal tweezers, Paster pipettes, Sterilizing discs, Bec Bunsen, Incubator	Samples, Muller Hiton agar (medium for antibacterial activity), Physiological saline, Nutrient agar

- **Bacterial strains:**

The bacterial strains used are Escherichia coli and Staphylococcus aureus.

- **Disk diffusion method:**

The antibacterial activity of the different membranes was evaluated using the disk diffusion method. The bacteria were inoculated on Mueller-Hinton agar poured into sterile Petri dishes. A small amount of the membrane like a dick was placed on the surface of the inoculated agar (in the middle of the Petri dish). The Petri dishes were then incubated (Figure III.14) at 37°C for 24 hours. A compound is considered active when an inhibition zone around the disk is measured with a diameter greater than 9mm, within which no bacterial growth is observed.

- **Principle**:

The principle of the method as mentioned in Figure II.12 is based on the diffusion of the antibacterial compound in a solid medium in a petri dish, creating a concentration gradient after a certain contact time between the product and the target microorganism. The effect of the antibacterial product on the target is assessed by measuring an inhibition zone, and depending on the diameter of the inhibition zone [111].

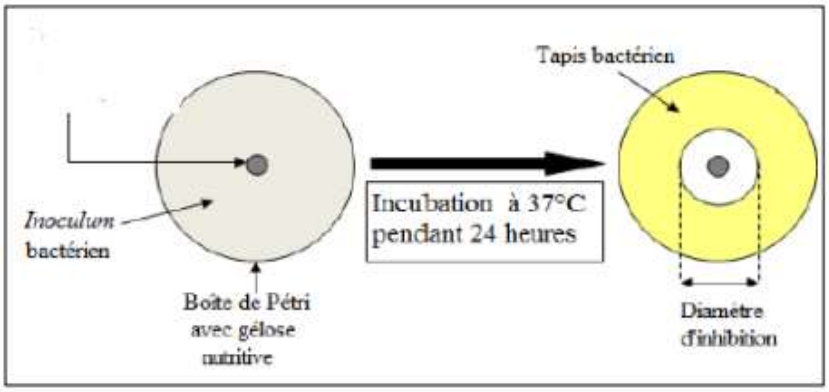


***Figure II. 12**: principle of the diffusion disk method*

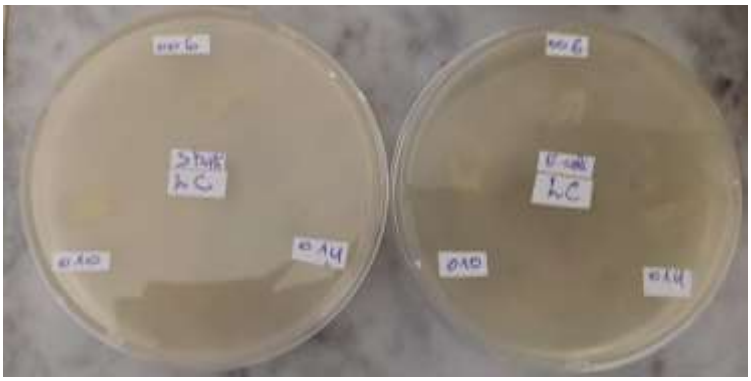


***Figure II. 13**: Petri dishes contain samples before the incubation*

***Figure II. 14**: Incubator contain the samples*

Chapter 03: Results and Discussion

This chapter present the different results obtained from various experiments conducted to the elaboration of certain membrane and the analyses of its characterization graphs (FTIR, DRX, UV).

1. Membranes obtained:

Biocatalytic nanocomposite membrane were successfully prepared using polyvinyl alcohol (PVA) and polystyrene sulfonate (PSS). A series of films was produced under identical conditions, varying only the mass fraction of the biocatalytic substance, which is prepared from the watercress and sesame seeds. A virgin PVA/PSS film was also prepared under the same conditions as the nanocomposite films to provide a basis for comparison. The prepared membranes appear as very thin films, approximately a hundred micrometers thick as Figure III.1 mentions.

000: PVA/PSS membrane, **LGC010**: PVA/PSS/watercress lipases, **LGS010**: PVA/PSS/ sesame lipase

***Figure III. 1**: images of the prepared membranes 000, LGC010, LGS010*

The elaboration of a membrane based on poly vinyl alcohol (PVA), poly sulfonated styrene (PSS), and a plant lipase requires a balance between the material fractions to ensure mechanical properties, enzymatic stability, and good catalytic activity. The optimal fraction depends on the specific objectives (permeability, selectivity, stability… etc.) and the experimental protocol. According to previous researches our fractions are determined based on these points, in the Table III.1.

- PVA is the main polymer serving as a matrix, providing good mechanical stability and compatibility with water.
- PSS is added to impart a negative charge to the membrane, which improves interaction with water and enzyme stability.

- The amount of lipase added should balance the enzyme activity and avoid agglomeration or denaturation.
- Crosslinking is essential to stabilize the membranes, so glutaraldehyde is added.

Table III. 1: Nomenclature and composite of the nanocomposite films prepared.

Nomenclature of nanocomposites films	PVA content (%)	PSS content (%)	Biocatalysis content (%)
PVA/PSS	80	15	0
PVA/PSS/LGC006	80	15	3
PVA/PSS/LGC010	80	15	5
PVA/PSS/LGC014	80	15	7
PVA/PSS/LGS06	80	15	3
PVA/PSS/LGS010	80	15	5
PVA/PSS/LGS014	80	15	7

It should be noted that:

- LGS lipase extracted from sesame seeds
- LGC lipase extracted from watercress seeds

2. Characterization of membranes

After the preparation of the membranes, several techniques were used to characterize them, namely textural analysis by XRD and qualitative analysis by FTIR.

2.1. XRD texture analysis

We chose to make this characterization for the lipases extracted from seeds in order to have an idea about the textural of it.

2.1.1. XRD of LGC :

The XRD pattern of LGC (Figure III.2) displays a broad and diffuse peak without sharp diffraction lines, which is characteristic of amorphous or low-crystallinity materials. This result aligns with the typical structural features of enzymatic or proteinaceous substances, which generally lack long-range crystalline order due to their complex and flexible macromolecular structures. The gradual increase in intensity across the 2θ range (approximately 2° to 10°) further confirms the amorphous nature of the extracted lipase.

This amorphous profile is advantageous for membrane applications, as it may facilitate better dispersion within the PVA matrix, enhancing enzyme immobilization efficiency and catalytic accessibility in biocatalytic membrane systems.

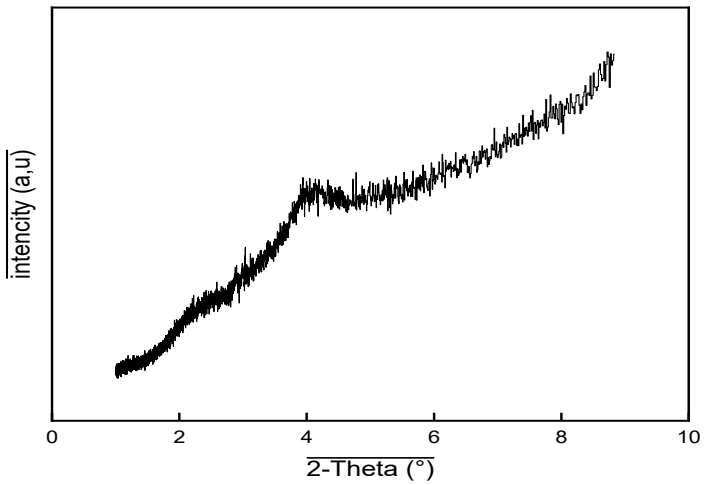


***Figure III. 2**: X-ray diffractogram of LGC.*

2.1.2. **XRD of LGS:**

The XRD spectrum diagram of the LGS is illustrated in Figure III.3. The pattern shows a broad peak around 21° (2θ), indicating that the lipase is amorphous and lacks a crystalline structure. This is typical for enzymes due to their complex and flexible nature. The amorphous state may enhance the enzyme's dispersion and interaction with PVA, aiding effective immobilization in membrane applications.

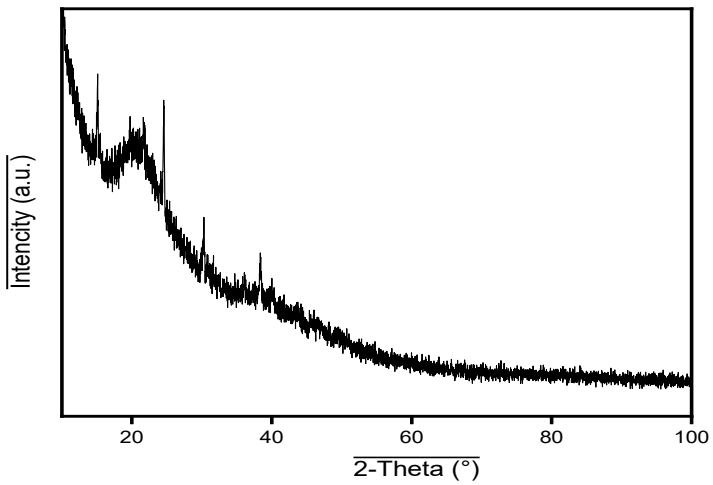


***Figure III. 3**: X-ray diffractogram of LGS.*

2.2. Characterization by FTIR infrared spectroscopy:

According to IR analysis, the identification of functional groups and the bonds between the chemical species that form the complex membranes can be carried out. The IR spectra of the different membranes developed, as well as the biocatalytic membranes, are presented below.

- **FTIR of 000 membrane:**

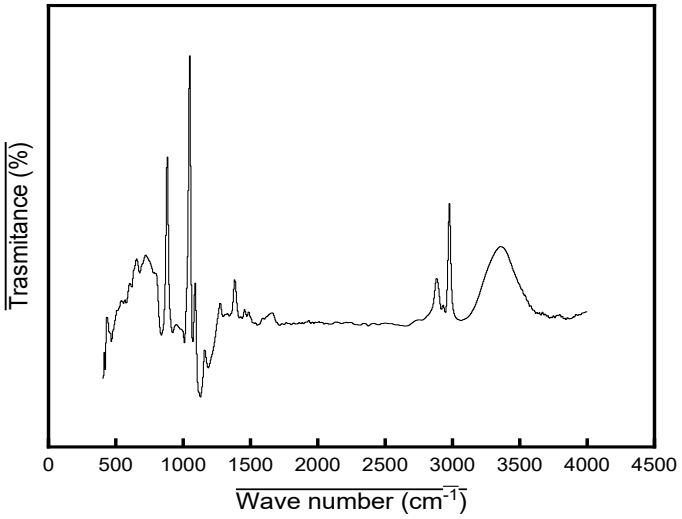


Figure III. 4: 000 membrane infrared spectrum.

Table III. 2: Main attribution of the FTIR bands of 000 membrane.

Wave number (cm^{-1})	Peak assignments
~ 3300 cm^{-1}	Confirms O-H groups in PVA
~ 2900 cm^{-1}	C-H aliphatic stretching in both polymers
1600-1500 cm^{-1}	Aromatic C=C from PSS
1200-1000 cm^{-1}	S=O from sulfonated groups in PSS and C-O from PVA
Fingerprint region below 900 cm^{-1}	Aromatic C-H bending

This IR spectrum (Figure III.4) consisted with a composite membrane made of PSS and PVA, and according to the table above (Table III.2) showing the presence of:

- Hydroxyl functionality (PVA)
- Aromatic and sulfonate features (PSS)
- No unexpected groups, suggesting no major contaminants.

- **FTIR of membranes contain LGC:**

This IR spectrum (Figure III.5) shows three biocatalytic membranes composed of PVA, PSS, and varying concentrations of lipases extracted from watercress seeds. With:

🔴 **LGC014 (Red):** Highest lipase content

🔵 **LGC010 (Blue):** Medium lipase content

⚫ **LGC006 (Black):** Lowest lipase content

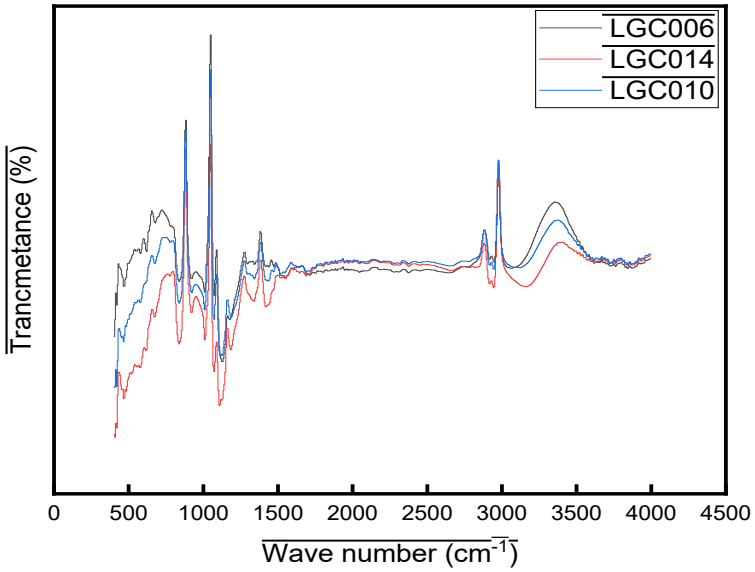


***Figure III. 5**: LGC membranes infrared spectrums.*

Table III. 3: Main attribution of the FTIR bands of LGC membranes.

Wave number (cm^{-1})	Peak assignments	Observation
~ 3200-3500 cm^{-1}	O-H groups in PVA and N-H stretching (amines, amides)	Red > Blue > Black intensity trend suggests increasing presence of enzyme (protein) due to more N–H and O–H groups.
~ 2900 cm^{-1}	C-H aliphatic stretching	From aliphatic $–CH_2$, $–CH_3$ in PVA and the protein backbone. Similar in all.
~ 1650 cm^{-1}	Amide I (C=O stretch in proteins)	Red spectrum shows the strongest peak here; confirms more protein (lipase).
~ 1540 cm^{-1}	Amide II (N-H bending + C-N stretch)	Another protein-specific band. Present more clearly in red and blue spectra than in Black.
~ 1180 – 1040 cm^{-1}	S=O stretching (PSS) + C-O stretching (PVA)	All samples have this.
Below ~ 900 cm^{-1}	Aromatic C-H bending (PSS)	No significant change with enzyme concentration

According to the Table III.3, the increase in lipase concentration enhances amide I and II bands (~1650 and 1540 cm^{-1}). Also, broadening/intensification of the 3300 cm^{-1} region aligns with higher protein content and potential hydrogen bonding with PVA/PSS. At the other hand there is no new peaks → suggests physical entrapment or mild interaction, not covalent bonding.

- **FTIR of membranes contain LGS:**

This IR spectrum (Figure III.6) shows three biocatalytic membranes composed of PVA, PSS, and varying concentrations of lipases extracted from sesame seeds. With:

🔴 **LGS014 (Red):** Highest lipase content

🔵 **LGS010 (Blue):** Medium lipase content

⚫ **LGS006 (Black):** Lowest lipase content

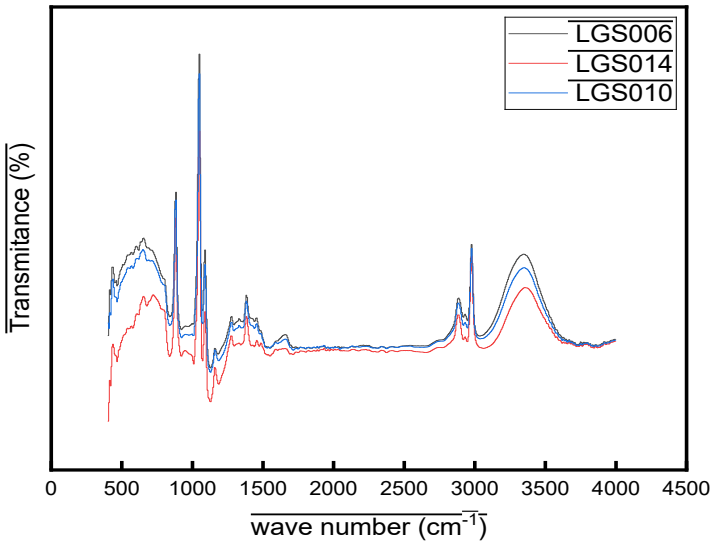


***Figure III. 6**: LGS membranes infrared spectrums.*

The latter shows that the higher enzyme content (LGS014 - red) leads to stronger Amide I and II bands, confirming greater protein presence. Also, the O–H/N–H stretching also increases with enzyme concentration, consistent with lipase structure. And with no significant shifts in PVA or PSS characteristic peaks, indicating that the enzyme is incorporated without major disruption to the base polymer structure.

Table III. 4: Main attribution of the FTIR bands of LGS membranes.

Wave number (cm^{-1})	Peak assignments	Observation
~ 3200-3500 cm^{-1}	O-H stretching vibration (from PVA, PSS, and possibly lipase proteins).	The intensity slightly increases with increasing lipase concentration (red > blue > black), suggesting a higher number of hydroxyl or amine groups from proteins in lipase.
~ 2900 cm^{-1}	C-H aliphatic stretching (common in PVA)	All spectra show similar features here, indicating that PVA content is consistent.
~ 1540-1650 cm^{-1}	Amide I (C=O stretching) and Amide II (N–H bending and C–N stretching), respectively.	These peaks are more intense in the red spectrum (LGS014), reflecting the proteinaceous nature of the lipase. This confirms the increasing presence of enzyme.
~ 1000 – 1200 cm^{-1}	S=O stretching of sulfonate groups from PSS.	These peaks remain relatively stable, supporting that PSS concentration is unchanged.
~840-920 cm^{-1}	C–O–C or PVA-related skeletal vibrations	similar for all spectra with minor differences due to lipase-polymer interaction.

- **FTIR Spectrum Comparaison : PVA/PSS/LGC vs. PVA/PSS/LGS**

Table III. 5: FTIR spectrum comparison between PVA/PSS/LGS membrane and PVA/PSS/LGC membrane

Aspect	LGC (Watercress)	LGS (Sesame)	Conclusion
Intensity of amide bands	Moderate	Higher	Sesame lipase seems richer in protein or more reactive with the matrix.
Hydrogen bonding (O–H/N–H)	Present and increasing	Strong and increasing	Suggests more polar functional groups or better enzyme dispersion in LGS.
PVA & PSS signals	Unchanged	Unchanged	Both systems maintain the structural integrity of the base polymer.
Spectral clarity	Less defined in some regions	Sharper peaks	LGS provides clearer fingerprint regions, suggesting more defined incorporation.

2.3. UV-visible characterization:

- **UV-vis of the membrane LGC006:**

The spectrum in Figure III.7 shows that the PVA–PSS–lipase membrane (curve B) has strong absorbance starting around 280 nm, due to aromatic groups in PSS and proteins from the lipase. The reference (curve C) shows low absorbance, confirming the successful incorporation of lipase into the membrane.

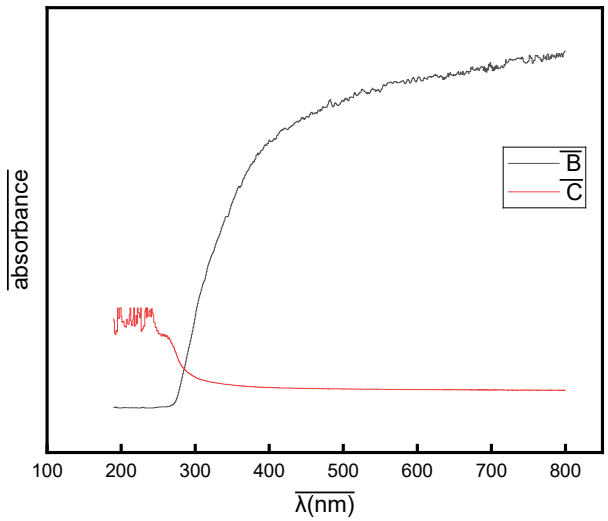


***Figure III. 7**: UV-vis of the membrane LGC006*

- **UV-vis of the membrane LGS006:**

The UV-Vis spectrum (Figure III.8) indicates that the membrane containing PVA, PSS, and sesame lipase (curve B) exhibits significant absorbance from around 280 nm, attributed to the aromatic groups of PSS and protein content from the lipase. The low absorbance of the reference membrane (curve C) confirms the effective incorporation of sesame lipase into the membrane.

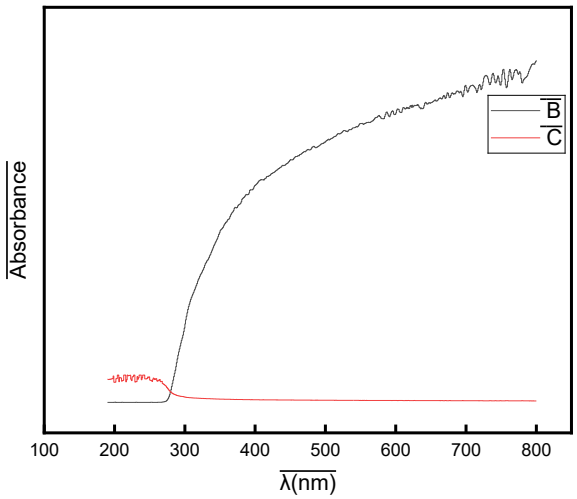


***Figure III. 8**: UV-vis of the membrane LGS006*

2.4. Antibacterial Activity Test:

The reading of this results is done by measuring the diameter of the inhibition zone around each sample of membrane.

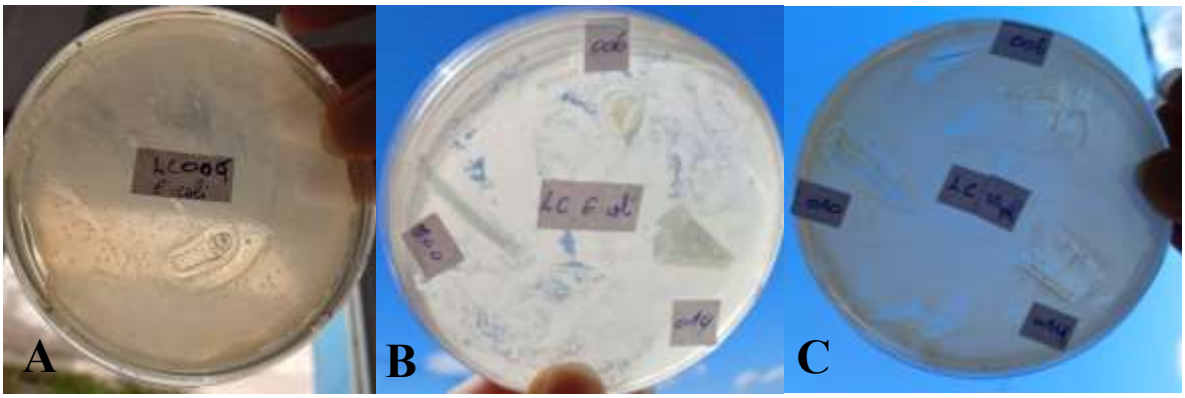


***Figure III. 9**: The results obtained from the antibacterial activity tests of LGC membranes*
A: LGC006 in E. coli - **B**: LGC006, LGC010 and LGC014 in E. coli - **C**: LGC006, LGC010 and LGC014 in staph

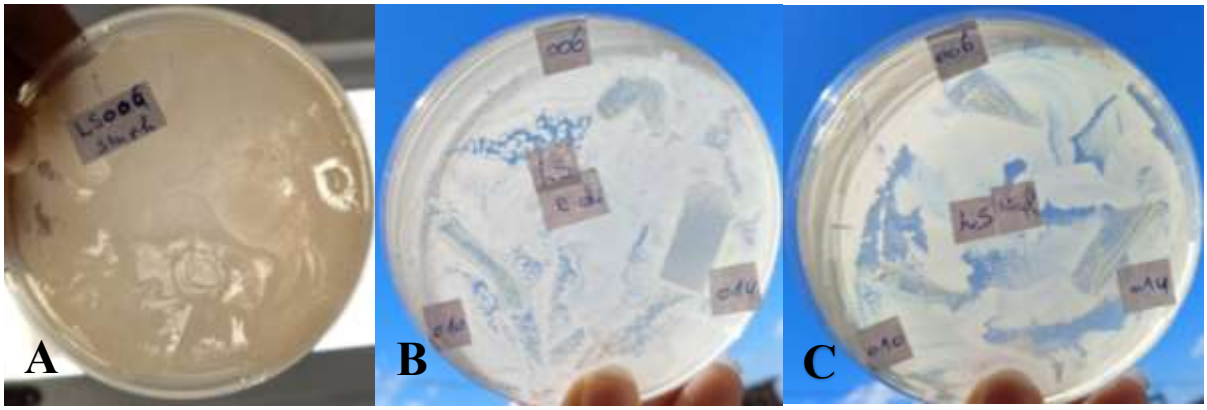


***Figure III. 10**: The results obtained from the antibacterial activity tests of LGS membranes*
A: LGS006 in E. coli - **B**: LGS006, LGS010 and LGS014 in E. coli - **C**: LGS006, LGS010 and LGS014 in staph

The results are positive for both type of membranes that contain LGC and LGS for both fractions 006 and 010 but for the fraction 014 there is no antibacterial activity may be its due to the accumulation of the protein. As a results we can conclude that both of membranes that contain LGC and LGS are resistance to Escherichia coli and Staphylococcus aureus but with small fraction, where 006 is the optimal and have an high antibacterial activity.

3. Conclusion

In this chapter, we mentioned the elaboration of the biocatalytic membranes by the use of PVA/PSS and both LGC and LGS. Seven membranes were successfully prepared from PVA/PSS and from different fractions of LGC and LGS. According to the results of the characterization techniques, the immobilization of lipases in PVA/PSS is confirmed successfully. while, LGC (watercress lipase) shows successful incorporation but with slightly lower intensity in key protein-related regions, the LGS (sesame lipase) membranes exhibit stronger and more defined amide peaks, indicating either: Higher protein content, better enzyme-polymer interaction or a more structurally defined enzyme. For the antibacterial test the LGS is the membrane that have the highest antibacterial activity.

General Conclusion

Biocatalytic membranes represent a significant advancement in membrane technology by integrating enzymatic or microbial catalysts within membrane structures. The objective of this work is the elaboration of a biocatalytic membranes, which is based on PVA and PSS.

Thus, we succeeded in producing seven membranes. The first one is without biocatalytic substance. The others are biocatalytic membranes; three of them contain LGC (which is a biocatalyst extracted from watercress seeds) with different fractions. The same protocol is applicated for lipase extracted from sesame seeds (LGS), where other three membranes are elaborated with varying the amount of LGS added. In order to determine the crystallinity and the phase composition of the lipases extracted, an X-rad diffraction test confirmed that this amorphous state may enhance the enzyme's dispersion and interaction with PVA, aiding effective immobilization in membrane applications.

Moreover, we used two techniques to characterize the membrane obtained, qualitative analysis by FTIR and UV-vis spectroscopy. The different results obtained allowed us to conclude that:

- the immobilization of lipases in PVA/PSS is confirmed successfully.
- LGS (sesame lipase) membranes exhibit stronger and more defined amide peaks in FTIR spectrum, indicating either: Higher protein content, better enzyme-polymer interaction or a more structurally defined enzyme.
- For the antibacterial test the LGS is the membrane that has the highest antibacterial activity.

Overall, this work contributes to the growing field of green and sustainable technologies, highlighting biocatalytic membranes as a promising tool for future industrial and environmental applications.

Many **perspectives** arise from this research, namely:

- Produce the enzyme under optimized conditions.
- Complete the study by considering the effect of other factors such as: temperature, and the optimal amount of enzyme.
- Optimization of extraction and purification of seed enzymes for maximal activity and yield.
- Long-term stability and reusability studies of the biocatalytic membrane under real operating conditions.
- More structural characterization for a better understanding enzyme distribution and interaction within the membrane.
- Kinetic studies to assess the effect of immobilization on enzyme activity.
- Application testing in relevant bioprocesses such as esterification, dye degradation, or pollutant removal.

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